ETHNOMEDICAL APPROACH FOR ERADICATION OF ORAL BIOFILMUSING EUPHORBIA HIRTA

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INTRODUCTION

Herbal medicine is gaining the upper hand in par with allopathic medicine in various disease management among human beings. Traditional medicinal plants and their products have been tested scientifically and are marketed to cure diseases in humans, as they are equally potent and less toxic with minimal side effects. Indian systems of medicine, such as Ayurveda and Siddha, recommend the use of plants as a cure from simple ailments to advanced diseases; 70% of the Indian population prefers traditional medicine [1]. The British colonisation of India had a complicated and multidimensional impact on traditional Indian medicinal systems including Ayurveda, Unani, and Siddha. Negative repercussions included the marginalisation of Indian medicine as it was perceived to be unscientific, discrimination in the availability of healthcare to different populations, and a neglect of research and development in Indian systems of medicine, all of which contributed to the downfall of Indian systems of medicine. Following independence, Indian systems of medicine experienced a revival for the following reasons: 1. A focus on holistic health, 2. Accessibility and affordability, 3. Natural remedies, 4. Scientific validation of traditional medicines, and 5. Increased public awareness of the benefits of Indian systems of medicine.

Oral diseases such as dental caries, gingivitis, and periodontitis are caused by pathogenic bacteria present in dental plaque, and the removal of dental plaque on a daily basis is recommended for the prevention of oral diseases [2]. In addition to chemical agents such as Chlorhexidine and Triclosan, dentifrices and mouthwashes often incorporate herbal extracts such as Neem, Aloe Vera, Clove oil, Tulsi, Salt, and Turmeric to eliminate dental plaque [3].

Many herbal extracts are still under review for use against oral pathogens. Euphorbia hirta is frequently known as “Asthma plant” in English and “Dudhi” in Hindi. This plant is broadly disseminated worldwide, with its primary presence in Asia. It can be found in countries such as Yemen, Oman, Palestine, Taiwan, Syria, Lebanon, India, Bhutan, Pakistan, Nepal, Myanmar, Thailand, Sri Lanka, Indonesia, Malaysia, Papua New Guinea, and Philippines. This plant belongs to the Euphorbia family of Euphorbiaceae. Euphorbia hirta has long served humanity in the form of

traditional and folk medicine. In addition to Euphorbia hirta, other species of the genus Euphorbia also show medicinal importance and are used in traditional medicine [4]. Euphorbia hirta is a high-value medicinal plant possessing significant antimalarial, antifungal, antifertility, antispasmodic, sedative, antiasthmatic, anthelmintic, anticancer, and antibacterial properties [5].

E. hirta is a very popular herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility and venereal diseases [6]. Moreover, the plant is also used to treat skin and mucous membranes, including warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, and Guinea-worm, and as an antiseptic to treat wounds, sores, and conjunctivitis. The plant has a reputation as an analgesic to treat severe headaches, toothaches, rheumatism, colic, and pain during pregnancy. It is used as an antidote for pain relief in scorpion stings and snakebites. The use of latex from Euphorbia Hirta to facilitate the removal of thorns from the skin is common [7].

Dental plaque is a diverse microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Once the tooth surface is cleaned, a conditioning film of proteins and glycoproteins is rapidly adsorbed onto the tooth surface. Plaque formation involves the interaction between early bacterial colonizers and this film (the acquired enamel pellicle) [8]. To facilitate colonization of the tooth surface, some receptors on salivary molecules are exposed to bacteria only once the molecule is adsorbed onto the surface. Subsequently, secondary colonizers adhere to the attached early colonizers (co-aggregation) through specific molecular interactions. These can involve protein-protein or carbohydrate-protein (lectin) interactions, and this process contributes to determining the pattern of bacterial succession. As the biofilm develops, gradients in biologically significant factors develop, permitting the coexistence of species that would be incompatible with each other in a homogenous environment. Dental plaque develops naturally but is also associated with two of the most prevalent diseases affecting industrialized societies (caries and periodontal diseases) [9].

Long term use of topical antimicrobials like Chorhexidine, Triclosan, Cetyl Pyridium Chloride, Alcohol etc., [10] by incorporating in toothpaste and mouthwash can result in development of resistance by the target microorganisms, hence there is a need for alternative topical antimicrobials, to overcome this issue, herbal products serve as a promising alternative, ‘Euporbia Hirta’ available as a common herb with antimicrobial, analgesic properties can serve as a good topical antimicrobial against dental plaque bacteria.

AIM AND OBJECTIVES

Aim

To determine the antimicrobial efficiency of Euphorbia Hirta

Objectives

- To determine the phytochemistry of Euphorbia Hirta
- To determine the antimicrobial efficiency of Euphorbia Hirta
- To determine the toxicity of Euporbia Hirta on Oral Epithelial Cells.

REVIEW OF LITERATURE

Asha S et al (2015) [11] conducted a study to identify the various phytochemical constituents present in the three different extracts of Euphorbia Hirta. Powdered plant material contained 8.90% Ash value, 7.0% w/w water soluble extract, 14.85% w/w ethanol soluble extract, 9.71% w/w methanol soluble extract, 9.84% w/w moisture content and fluorescence to some treatments. The chemical constituents of the ethanolic and methanolic leaf extracts of the plant were relatively similar in the presence of proteins, fats and oils, gums and mucilages as primary metabolites and alkaloids, flavonoids, terpenoids, tannin, phenol, steroid, glycoside, saponin, coumarin as secondary plant metabolites. Amino acids, carbohydrates and anthraquinones did not detected. In contrast, aqueous extract appeared to posses the constituents such as carbohydrates, proteins, amino acid, tannin, phenol, steroid, saponin and anthraquinone with a negative result to alkaloids, flavonoids, terpenoids, glycosides, fat and oils, coumarin, gums and mucilages. In conclusion, the reported results support the leaf extracts of *Euphorbia hirta* to exhibit a wide spectrum of pharmacological activities and promisingly used as traditional medicine.

Abu Arra Basma et al 2011 [12] conducted a study to assess the antioxidant activities of Euphorbia hirta by diphenyl -1- picrylhydrazyl (DPPH) assy, The leaves extract exhibited a maximum DPPH scavenging activity of (72.96±0.78)% followed by the flowers, roots and stems whose scavenging activities were (52.45±0.66)%, (48.59±0.97)%, and (44.42±0.94)%, respectively. Phytochemical screening of *E. hirta* leaf extract revealed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, flavanoids and phenolic compounds. These results suggeste that *E. hirta* have strong antioxidant potential.

Kuntal Das et al 2022 [13] conducted this study to assess the phytochemical properties and in-vitro anti-inflammatory activities of lea and roots of Euphorbia hirta. Dried Euphoria Hirta leaves and roots were subjected to Soxhlet extraction under ethanol. Bioactive compounds alkaloids, glycosides, flavonoids, tannins, and phenolics were reported in leaf and root extracts. Ethanol EH leaf extract exhibited significant anti-inflammatory activity than root by inhibiting albumin denaturation, proteinase, and the lipoxygenase activities with 87.51 %, and 51.2 % followed by

97.30 %, and 54.21 % followed by 94.43 % and 48.21 % respectively at 100 µg/ml concentration. The results affirmed that ethanol EH leaf extract showed better anti-inflammatory activity than root extracts.

Waseem Ahmed et al 2017 [14] conducted the present study to investigate the phytochemical screening and antimicrobial activities of Euphorbia hirta extracts. Phytochemical screening revealed the presence of alkaloid, flavonoid, saponin, terpenoid, steroid and sterols in the extracts of aerial part of Euphorbia hirta. Methanol, chloroform and hexane extract of leaf and fruit were tested against Proteus mirabilis, Listeria monocytogenes, Clostridium absonum, Aspergillus niger, Aspergillus fumigates, Arthrographis cuboidea by the agar disc diffusion method.

Upadhyay B et al [2010] [15] conducted a study to determine the antibacterial activity of Euphorbia Hirta against many Gram positive and Gram negative bacteria (Standard strains and clinical isolates) using disc diffusion method. Antibacterial sensitivity test indicated that the methanolic extract inhibited the growth of S. aureus, E. coli, and B. subtilis to varying extents while K. pneumonia was the most resistive strain to these extracts. Minimum inhibitory concentration (MIC), of the extract against E. coli, S aureus, and S. entertidis were in the range of 0.1mg/ml. Phytochemical analysis indicates presence of terpenes, tannins, alkaloids and flavonoids which might be accountable for its antimicrobial properties, and these results validate the traditional uses of the plant in the treatment of various diseases.

Iskandar B et al 2022 [16] conducted this study to formulate a *Euphorbia hirta L.* ethanol extract for mouthwash, evaluate the physical properties, and determine its anti-bacterial effects against *Streptococcus mutans*. Each mouthwash formula was created by utilising a solubilisation technique. Three mouthwash formulas were created from different concentrations of *Euphorbia hirta L.* ethanol extract (0.5%, 1%, and 2%). 2% mouthwash showed that in lower contact time with a neutralisation of 57.14% of *Streptococcus mutans* colonies in 30 seconds, and showed stable anti-bacterial control against *Streptococcus mutans* ($p = 0.000$) within the first week of use.

Jackson C et al (2009) [17] conducted an in vitro study to assess effects of Nystatin and methanol extract of leaves of Euphorbia hirta against clinical isolates of

Candida albicans were investigated using the Checkerboard technique. The organism was susceptible to the extract with MIC of 25 mg/ml, while Nystatin had MIC of 0.165 mg/ml against C. albicans. The results indicate that combinations of the extract with nystatin could be synergistic in activity for some ratio combinations and indifferent for some others.

Rajeh MA et al (2012) [18] conducted a study to assess *In vivo* brine shrimp lethality assay and oral acute toxicity study at single high dose of 5000 mg/kg and observation for 14 days in mice were used to study the toxic effect of *E. hirta*. Brine shrimp lethality assay was used to calculate the median lethal concentration (LC_{50}) of *E. hirta* (for leaves, stems, flowers and roots) methanolic extracts at concentrations from 100 to 0.07 mg/ml. The LC_{50} values of 1.589, 1.420, 0.206 and 0.0827 mg/ml were obtained for stems, leaves, flowers and roots, respectively. *E. hirta* leaves methanol extract has exhibited mild toxic effects in mice.

Ping KY et al (2013) [19] conducted a study to assess cytotoxicity and genotoxicity activity of *Euphorbia hirta* (*E. hirta*) in MCF-7 cell line model using comet assay. The extract of *E. hirta* showed significant toxicity against brine shrimp with an LC_{50} value of 620.382 µg/mL (24 h). The present work confirmed the cytotoxicity and genotoxicity of *E. hirta*.

Pingale S et al (2013) [20] investigated the acute toxicity of Euphorbia hirta plant material in Swiss mice. The plant material was administered orally at dose ranging from 2gm to 10gm/Kg body weight of the plant material in the form of slurry. The animals were observed continuously for the first 4 hours for any behavior changes and they were then kept under observation up to 14 days after administration of the plant material as drug to find out the mortality. From the observations it is concluded that Euphorbia hirta plant material at doses of 10 g/kg body weight is nontoxic since no marked changes in behavioral, food and water intake were observed. The Euphorbia hirta plant material was found to be safe. No lethality or adverse toxic signs were seen during the experimental study period and during 14 days observation period. No delayed toxic signs were reported in all experimental groups. These findings of this study suggest that the Euphorbia hirta could be relatively safe when administered orally in Swiss mice.

MATERIALS AND METHODS

An *in-vitro* study was carried out to determine the phytochemisty, minimum inhibitory concentration (MIC) of ethanolic extract of *Euphorbia hirta* (*E. hirta*) against common oral pathogens and toxicity of the extract on oral epithelical cells. Approval has been obtained from the Institutional Scientific Review Board of Asan Memorial Dental College & Hospital (AMDECH/ISRC/2021/03 - 010).

Identification of Plant

Euphorbia hirta plant [Tamil Name – Amman Pacharisi] was obtained from the herbal farm run by a Non- Governmental Organization named ITWWS – Irular Tribe Women's Welfare Society, Thandarai, Chengalpet District, Tamilnadu, India. The plant was authenticated by the Department of Botany, Madras Christian College, East Tambaram, Chennai, India.

Preparation of Plant Extract

The collected plant was washed with distilled H_2O and the leaves were shade dried for a period of 7 to 10 days. The dried plant leaves were powdered in electrical mill to 60 – mesh size. Ethanolic extract was obtained using Soxhlet method as described by Jensen SB [21]. Briefly, 20 g of powdered sample was placed in the thimble, kept Soxhlet extractor using ethanol as solvent. The solvent is been heated in a mantle and vapours, pass to the condenser. Condensed vapours drip into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. Cycle was repeated for 48 hours, temperature was maintained at 75^0C. The filtered extract was dried using rotary flash evaporator in hot condition (40^0C). The crude extracts were further dried with vaccum concentrate and the extract was stored at 4^0C for further analysis.

GCMS Analysis

The extract was concentrated using oven at 35 C to obtain dried extract it was analyzed separately by GC-MS using a HP-5MS capillary column (30 m ˆ 250 µm, i.e., 0.25 µm film thickness) in an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a water GCT Premier mass spectrometer (Waters Corporation, Milford, MA, USA). The carrier gas was helium

with a constant flow rate of 3 ml/min. The oven temperature was initially kept at 70 C for 3min then ramped at 10*C/min to 240*C. The temperature was gradually increased from 8 *C/min to 300*C and held isothermally for 9 minutes. An amount of 1.0 µL of the sample solutions was injected in the splitless mode. Mass spectra were obtained over the scan range m/z 40 -600. The compounds were identified by comparison of their mass spectra with those of the NIST 05 L mass spectral library. The spectral match factor limit was set at 600 and any components with match factor less than 600 were not considered. GC – MS Analysis is been conducted at Bureau veritas Laboratory, Ekkatuthangal, Guindy, Chennai accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL).

Collection of microbial strain

Streptococcus mutans (ATCC – UAB577), *Escherichia coli* (SDC – 08), *Candida albicans* (SDC – 03) were gifted by Dr. Sankar Ganesh, Department of Microbiology, Saveetha Dental College & Hospital and *Lactobacillus Acidophilus* (MTCC – 10307) was procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Preparation of inoculums *and C. albicans*

The selected bacterial cultures (*S. mutans, L acidophilus, E. coli* and *C. albicans*) were sub-cultured in BHI agar. A loop full of culture was transferred into the freshly prepared BHI broth and the tubes were incubated at 37^0C for 18h. After incubation, 0.5 standard McFarland solutions (1.5×10^8CFU/ml) were prepared with bacterial and fungal culture. These standard solutions were further used for antibacterial susceptibility tests.

Determination of MIC

Broth dilution method was carried out to determine the MIC using broth dilution method. The MIC of *Euphorbia hirta* against *Streptococcus mutans, Lactobacillus acidophilus, Escherichia coli and Candida albicans* were determined. 10µl of overnight culture were added to the Brain Heart Infusion Broth with ethanol extract of *Euphorbia hirta* ranging from 0.01 – 10 mg/ml (two fold serial dilutions) and without extracts (Control). The tubes were incubated at 37^0C for 24 h. The growth of bacteria was visualized by the addition of 10 µl of 2,5 – triphenyl terazolium

chloride (0.1mg/ml) solution. The lowest concentration at which there was no visible growth was recorded as MIC.

Anti-microbial effect of Euphorbia Hirta extract is been carried out at the Orange Lab, Department of Microbiology, Saveetha Dental College & Hospitals, Chennai.

Toxicity Assessment - MTT assay

Toxicity assessment of the extract is been carried out at Molecular Biology Laboratory, Saveetha Dental College & Hospital. Primary human oral epithelial cells (OECs) were cultured as previously described Lee et al., 2017 [22] & Hyun et al., 2019 [23]. The human epithelial cells were cultured in respective media in 96-well plates to ~ 80% confluence and the cells were treated with various concentrations of *Euphorbia hirta* extract, ranging from 0 to 300µg for 24 h. Next, the media was aspirated to wash the cells with phosphate buffer Saline (PBS). MTT solution at 0.5 mg/ml was added to each well and the plate was incubated at 37 °C for 4 h in the dark following which the MTT solution was replaced with 200 µl of DMSO. The plate was shaken at 150 rpm for 5 min and then the absorbance was recorded at 570 nm by an enzyme-linked immunosorbent assay reader and the results was analyzed in triplicate and the percentage was calculated. The cell viability is calculated using the formula

$$\%\,\text{Growth inhibition} = \left[\frac{A_{570}\ \text{nm of treated cells}}{A_{570}\ \text{nm of control cells}}\right] \times 100$$

RESULTS

GC-MS Analysis

GC-MS analysis of Ethanolic extract of Euphorbia Hirta revealed the presence of following phytochemicals depicted in Table -1. Constituents like Phytol and Octadeatienoic acid and Squalene possess antioxidant activity, anti-inflammatory response is been observed with hexadeconoic acid and hexacosane, Anti-microbial effect is been present due to Heneicosane, Octadecdienoic acid and Tetracosane.

Table 1: Biological activities of the phytochemical constituents of Euphorbia Hirta

S. No	Phytochemicals	Biological Activity
1	Phytol	Antioxidant
2	Diethyl phthalate	Plasticizer for film coating on tablets
3	9,12,15 Octadecatrienoic acid	Antioxidant
4	9-Octadecenamide	Antilipidemic
5	3,7,11,15 Tetramethyl2hexa decenol	Antioxidant, Anti-microbial
6	Hexadeconoic acid	Anti-inflammatory, Antioxidant
7	methylester n-Hexadeconoic acid	Anti-inflammatory, Antioxidant
8	9,12, Octadecadienoic acid	Anti-microbial, Antilipidemic Anti-cancer
9	Squalene	Anti-cancer, Antioxidant
10	Heneicosane	Anti-microbial, Pheromone
11	Hexacosane	Anti-inflammatory
12	2-methyloctacosane	Anti-microbial

13	Stigmasta 3-5 diene	Free radical scavenging, Anti-diabetic, Anticancer
14	Tetracosane	Antimicrobial, Anticancer
15	Hentriacontane	Analgesic
16	Vitamin E	Antioxidant
17	Stigmasterol	Thyroid Inhibition, Anti-peroxidative & Hypoglycemic
18	Gamma sitosterol	Anti-diabetic, Anti-depressant
19	Beta sitosterol	Anti-bacterial
20	Alpha amyrin	Analgesic, Anti-Inflammatory, Anti-depressant, Antilipidemic
21	Beta amyrin	Analgesic, Anti-Inflammatory, Anti-depressant, Antilipidemic
22	Urs 12 en24 oic acid 3 oxo methyl ester	Antiplasmodial activity

Antimicrobial Effect – Determination of Minimum Inhibitory Concentration (MIC)

Table – 2- depicts the Minimum Inhibitory Concentration (MIC) of Euphorbia Hirta ethanoloic extract on common oral pathogens, All the test organisms were susceptible to Euphorbia hirta extract at different concentrations mentioned below, Streptococcus mutans was susceptible at 0.15 mg/ml, Lactobacillus acidophilus was susceptible at 5 mg/ml, E Coli was susceptible at 1.25 mg/ml and Candiada albicans was susceptible at 0.312 mg/ml.

Table 2: Minimum Inhibitory Concentration (MIC) of Euphorbia Hirta

Organism	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**	**0.625 mg/ml**	**0.312 mg/ml**	**0.156 mg/ml**	**0.078 mg/ml**	**0.039 mg/ml**	**0.019 mg/ml**
S. mutans	MIC	MIC	MIC	MIC	MIC	MIC	MIC	G	G	G
L. acidophilus	MIC	MIC	G	G	G	G	G	G	G	G
E. coli	MIC	MIC	MIC	MIC	G	G	G	G	G	G
C. albicans	MIC	MIC	MIC	MIC	MIC	MIC	G	G	G	G

MIC – Minimum Inhibitory Concentration**, G** – Growth Observed

Toxicity Assessment of Euphorbia Hirta Extract

MTT assay method was employed to assess the toxicity of Euphorbia Hirta on oral epithelial cells, Table – 3 depicts the cell viability of oral epithelial cells, where it shows that there is proliferation of oral epithelial cells on increasing the concentration of the extract and there is no toxicity observed in cell viability.

Table 3: Cell viability of Oral Epithelial Cells using MTT Assay

Euphorbia hirta extract						
Control	50ug	100ug	150ug	200ug	250ug	300ug
100%	97.77%	104.44%	122.22%	135.55%	151.11%	168.88%

DISCUSSION

Oral biofilm is been a source for common dental diseases like caries and periodontal disease, the bacterial colonies in oral biofilm have a potency to affect different organ systems of human body, hence plaque control measures is been given utmost importance in maintenance of good oral hygiene. Plaque control measures like brushing, flossing and use of mouthwashes is been recommended to eliminate dental plaque, various chemical substances were added to the dentifrice like Chlorhexidine, & Triclosan were been added to the toothpaste for effective plaque removal.

However these substances have tendency to cause stains over the tooth surface and may cause antibiotic resistance on long term use, hence herbal products can be considered as potential alternatives, Neem, Tulsi, Aloevera, Cloves & Turmeric, were added to the toothpaste and are successful in supporting removal of dental plaque, Euphorbia Hirta is a common weed grown in many parts of our country was considered for oral biofilm removal in the current study.

Phytochemical analysis of Euphorbia Hirta revealed the presence of 22 different compounds of biological importance in the E hirta ethanol extract by GCMS. Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols [24]. It is the product of chlorophyll metabolism in plants; hence, phytol is abundantly available in nature. Phytol is known to inhibit the growth of *Staphylococcus aureus* [25] and to block the teratogenic effects of retinol [26]. 9,12,15- Octadecatrienoic acid is found to show antioxidant effect [27].

Squalene is a linear triterpene synthesized in plants, animals, bacteria, and fungi as a precursor for the synthesis of secondary metabolites such as sterols, hormones, or vitamins. It is a carbon source in the aerobic and anaerobic fermentation of microorganisms [28]. Scientific research has shown that SQ reduces skin damage by UV radiation, LDL levels, and cholesterol in the blood, prevents the suffering of cardiovascular diseases, and has antitumor and anticancer effects against ovarian, breast, lung, and colon cancer. [29]

Heneicosane is one of the major components of the safflower flower essential oil (Carthamus tinctorius) [30], identified in the current study in the ethanolic extract of Euphorbia Hirta it possesses antimicrobial action against various microorganisms. [31]

Hexacosane is a straight-chain alkane comprising of 26 carbon atoms. It has a role as a volatile oil component and a plant metabolite. [32] Hexacosane possess anti-microbial action against Klebsiela pnemoniae, Salmonela typhi, Mithecithinne staphaureus and Proteus vulgaris.[33]

2-Methyloctacosane, also known as isononacosane, belongs to the class of organic compounds known as branched alkanes. 2-Methyloctacosane is a very hydrophobic molecule, practically insoluble (in water), and relatively neutral. [34] It possesses antimicrobial activity [35].

Free radicals are unstable molecules that are made during normal cell metabolism (chemical changes that take place in a cell). They can build up in cells and cause damage to other molecules. This damage may increase the risk of cancer and other diseases. [36] Stigmasta 3-5 diene possess free radical scavenger action [37]. Tetracosane, also called tetrakosane, is an alkane hydrocarbon, it possess antimicrobial and anticancer effect [38].

Hentriacontane, also called untriacontane, is a solid, long-chain alkane hydrocarbon. hentriacontane was shown to ameliorate the expression of inflammatory mediators (TNF-α, IL-6, PGE(2), COX-2 and iNOS) and the activation of NF-κB and caspase-1 in LPS-stimulated peritoneal macrophages. [39]

Vitamin E (alpha-tocopherol), the principal chain-breaking antioxidant in biological membranes, prevents toxicant- and carcinogen-induced oxidative damage by trapping reactive oxyradicals. [40]. Vitamin E is found to be in high concentration in the extract.

Stigmasterol is a 3beta-sterol that consists of 3beta-hydroxystigmastane having double bonds at the 5,6- and 22,23-positions. It has a role as a vitamin and a plant metabolite. [41]. Stigmasterol possess Thyroid inhibitory, antiperoxidative and hypoglycemic effects [42]

γ-sitosterol has been reported for the first time in Girardinia heterophylla and has potential to be used as an antidiabetic owing to its remarkable medicinal properties while β-sitosterol is the common sterol found in almost all plants which has 5 double bond and α- ethyl at C-24. [43]

α and β-amyrin, is a pentacyclic triterpene that is isolated from the resin of the traditional medicinal plant, *Protium heptaphyllum*. α and β-amyrin has anti-inflammatory, antinociceptive, gastroprotective and hepatoprotective properties [44] is noted in the extract of euphorbia hirta in our study. Urs-12-en-24-oic acid, 3-oxo-, methyl ester [45], found in the extract possess antiplasmodial activity [46].

Ethanolic extract of Euphorbia hirta is found to be effective against oral pathogens, In the present study the minimum inhibitory concentration was 0.156 mg/ml similar to the study by W Ahmed et al (2020) [47] where similar effect is been observed with the ZnO nanoparticles of Euphorbia Hirta. Lactobacillus Acidophilus is responsible for progression of dental caries, Euphorbia Hirta extract had MIC value of 5 mg/ml against Lactobacillus acidophilus, which is higher than Tulsi extract 100 mg/ml in the study conducted by A Gadiyar et al (2017) [48].

E Coli is commonly associated with gingivitis and periodontal diseases [49], the ethanolic extract was effective against E Coli at the concentration of 1.25 mg/ml, lower than the MIC of aqueous extract of Euphorbia Hirta 50 mg/ml in the study conducted by EM Abubakar (2009) [50]. Euphorbia hirta extract used in the present study possessed anti-fungal effect against Candida albicans at 0.312 mg/ml, lower than the study conducted by Clement Jackson et al (2009) [51] where the MIC value was 25mg/ml against Candida albicans.

Oral epithelial cells serve as a protective barrier protecting the underlying mucosa from various microbioms and trauma occurring in the oral cavity. Herbal products like tulsi, neem, aloe vera are non-toxic to oral epithelial cells, MMT assay was carried out to assess the toxicity of Euphorbia hirta on oral epithelial cells showed that, the extract resulted in proliferation of cells in the present study there is no viability of cells observed.

CONCLUSION

- Euphorbia hirta possess anti-microbial and anti-inflammatory properties.
- Euphorbia hirta possess anti-microbial effect on common oral pathogens like Streptococcus mutans, E Coli, Lactobacillus Acidophilus and Candida Albicans.
- Euphorbia hirta is non-toxic to oral epithelial cells.

Future Scope

- Euphorbia Hirta is found to be beneficial against plaque microorganisms.
- Euphorbia Hirta can be considered to be added as an anti-microbial agent in dentifrices and mouthwashes.
- Formulation can be created for a dentifrice / Mouthwash using Euphorbia hirta based on the results of the current study.

FORMULATION OF EUPHORBIA HIRTA MOUTHWASH

Constituent	**Concentration**	**Role**
Ethanolic extract of Euphorbia Hirta	5%	Anti-microbial, Analgesic, Anti-fungal and Anti-Inflammatory Effect [Active Ingredient]
Sodium Benzoate	1%	Preservative
Propylene Glycol	20%	Retain Moisture
Stevia	5%	Sweetening Agent
Peppermint oil	2%	Flavouring Agent
Sodium Fluoride	0.2% [900 PPM]	Anti-Caries Agent
Water	66.8%	Vehicle (Solvent)

Euphorbia Hirta novel herbal mouthwash composition comprises a synergistic blend of ethanolic extract of Euphorbia Hirta (5%), sodium benzoate (1%), propylene glycol (20%), stevia (5%), peppermint oil (2%), sodium fluoride (0.2%), and water (66.8%). The ethanolic extract of Euphorbia Hirta serves as the primary active ingredient, exhibiting antimicrobial, analgesic, anti-fungal, and anti-inflammatory properties. Sodium benzoate acts as a preservative, while propylene glycol aids in moisture retention. Stevia provides sweetness without artificial additives, and peppermint oil imparts a refreshing flavour. Sodium fluoride is included for its anti-caries benefits. The composition offers enhanced oral hygiene benefits compared to conventional mouthwashes, providing antimicrobial, anti-inflammatory, analgesic, and anti-fungal effects while freshening breath and preventing tooth decay.

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